Does Methylmercury Have a Role in Causing Developmental Disabilities in Children?

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Methylmercury (MeHg) is a potent neurotoxin that in high exposures can cause mental retardation, cerebral palsy, and seizures. The developing brain appears particularly sensitive to MeHg. Exposure levels in pregnant experimental animals that do not result in detectable signs or symptoms in the mother can adversely affect the offspring's development. Studies of human poisonings suggest this may also occur in humans. Human exposure to MeHg is primarily dietary through the consumption of fish: MeHg is present in all fresh and saltwater fish. Populations that depend on fish as a major source of dietary protein may achieve MeHg exposure levels hypothesized to adversely affect brain development. Increasing mercury levels in the environment have heightened concerns about dietary exposure and a possible role for MeHg in developmental disabilities. Follow-up studies of an outbreak of MeHg poisoning in Iraq revealed a dose–response relationship for prenatal MeHg exposure. That relationship suggested that prenatal exposure as low as 10 ppm (measured in maternal hair growing during pregnancy) could adversely affect fetal brain development. However, using the same end points as were used in the Iraq study, no associations have been reported in fish-eating populations. Using a more extensive range of developmental end points, some studies of populations consuming seafood have reported associations with prenatal MeHg exposure, whereas others have found none. This paper reviews the data presently available associating MeHg exposure with development and poses some of the unanswered questions in this field. *Key words*: child development, developmental disability, fish consumption, methylmercury. — *Environ Health Perspect* 108(suppl 3):413–420 (2000). *http://ehpnet1.niehs.nih.gov/docs/2000/suppl-3/413-420myers/abstract.html*

Mercury is a heavy metal that is widely distributed in the earth's crust. Both natural and anthropogenic sources contribute to the global cycling of this element (*1*). In aquatic environments, inorganic mercury is converted to methylmercury (MeHg) by methanogenic bacteria present in sediments of fresh and oceanic water. The MeHg is then bioaccumulated and bioconcentrated as it passes up the aquatic food chain. All fish contain some MeHg and vertebrates (fish and sea mammals) at the top of the food chain contain the largest quantities.

Human Effects of Methylmercury

MeHg *in vitro* inhibits microtubule formation and protein synthesis in nerve cells, alters neuronal membrane activity, and interferes with DNA synthesis (*1*); *in vivo* it impairs mitosis and disrupts neuronal migration (*2,3*). Its toxicity has been known for centuries (*4*) and multiple episodes of poisoning in children by both inorganic and organic mercury have been reported (*5,6*). The organic forms of mercury are particularly neurotoxic (*7–16*). Both prenatal and postnatal exposure to MeHg can adversely affect the central nervous system, but it appears to be most neurotoxic prenatally when the brain is developing rapidly. Exposure to sufficient amounts can cause neurological impairment or even death (*1*). However, the lowest level of exposure that can produce health effects detectable using epidemiological methods is presently unknown.

Exposure Data from Poisoning in Japan

Only a few of the many MeHg poisoning episodes have advanced our understanding of the relationship of exposure to observable developmental disability (*7,17–19*). Industrial pollution led to MeHg poisoning in Minamata and Niigata, Japan, during the 1950s and 1960s. At Minamata, more than 2,000 people who consumed contaminated fish were officially recognized as being poisoned (*20*). These episodes provided information about the dangers of pollution and the devastating impact of MeHg poisoning at all ages. A Swedish expert group (*21*) reviewed the Minamata and Niigata poisonings in detail and provided an outstanding review of the data available on MeHg in fish. Only limited information about the exposure levels that might have caused disabilities was obtained in Japan. At Minamata, several years elapsed between the recognition of patients with neurological symptoms and identification of the causative agent (*22*). In addition, the exposure data available were difficult to interpret because the timing of specimen collection was often unclear and the method of analysis and its accuracy were not reported. Consequently, no association between exposure level and clinical symptoms was possible (*21*). At Niigata, MeHg poisoning was identified earlier and data on exposure levels in blood and hair as they related to the onset of symptoms were obtained (*21,23*). The Swedish expert group determined that the mercury level in hair at the onset of the disease was generally > 200 ppm, although the level in one patient may have been as low as 50 ppm.

After the epidemic at Minamata, Harada (*7*) identified 22 children who were exposed to MeHg *in utero* when their mothers consumed contaminated fish and who had developmental disabilities. Thirteen of the mothers had no symptoms of MeHg exposure during pregnancy, five complained of paresthesias, three of fatigue, and one of hyperemesis. All of the children had severe disabilities including mental retardation, cerebral palsy, and seizures. Exposure levels were measured in hair from 1 to 6 years after birth and ranged from 6 to 100 ppm in the children and from 2 to 191 ppm in the mothers. Harada (*7*) attributed the disabilities to the prenatal MeHg exposure and applied the term congenital Minamata disease.

After the Minamata epidemic only a few studies of children with less serious disabilities were reported. Harada (*24*) studied 223 schoolchildren 13–16 years of age in the Minamata area and compared them with 196 controls. He reported a significantly higher frequency of intellectual deficiency (18 vs. 7%), sensory disturbances (21 vs. 3%), and speech disorders (12 versus 2%) in children from the Minamata area than in controls. Interpreting these findings is difficult because individual exposures were not known and details of the survey methods were not reported. Other surveys were done but are also difficult to interpret (*25,26*).

At Niigata, the causative agent was identified earlier and some exposure levels were determined closer to the time of the poisoning. At Niigata, mothers who had hair mercury levels during pregnancy of > 50 ppm were given the option to terminate the pregnancy (*22,23*). At least 47 pregnant women exposed to MeHg were identified. Thirteen women with hair levels > 50 ppm elected to continue their pregnancy. Only one child was

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diagnosed with congenital Minamata disease at Niigata (*23*). That child had an uncle diagnosed with Minamata disease just before her birth. The mother's hair mercury level was 293 ppm and the child's hair mercury level was 77 ppm, but the timing of specimen collection was not clear. That child also received breast milk for the first 2 months of life, a potential source of postnatal exposure. It is difficult to determine the relationship between exposure and outcome from the Japanese data, but clearly children with severe disabilities were born to mothers with minimal evidence of poisoning.

Exposure Data from Poisoning in Iraq

In the winter of 1971–1972 there was a large outbreak of MeHg poisoning in Iraq. A number of circumstances led to rural villagers consuming MeHg-treated seed grain intended for planting. The source and cause of the poisoning was identified almost immediately and the government acted quickly to inform the public and stop the exposure. Despite this there were 6,530 hospital admissions and 459 hospital deaths attributed to MeHg poisoning (*19*). Because only the sickest patients were admitted and much of Iraq is rural, with limited access to health care, the number of individuals affected may have been substantially higher (*27*).

The Iraq poisoning differed in three important ways from the poisonings in Japan. First, exposure was limited to a short time interval; second, the causative agent was identified very early; and third, the exposure was accurately measured using new techniques including cold vapor atomic absorption. These differences resulted in valuable information being obtained in Iraq on the association between exposure and outcome.

Many children had either prenatal or postnatal poisoning, and some experienced both. The clinical picture in these children was confirmed as similar to that reported in Japan (*8–10,12–14*). Mercury levels in maternal hair growing during pregnancy were determined and, for the first time, assessment of associations between the level and timing of exposure and the children's disabilities was possible, thus providing a dose–response relationship (*15*). Mercury measured in maternal and child blood and maternal breast milk provided information about the relationships of mercury in different biological compartments (*10,13*). In addition, the effects of prenatal MeHg on the developing brain were examined in children who died (*2*).

More than 80 infants who were *in utero* during the time the MeHg-treated grain was being consumed by the mothers were examined and their prenatal exposure determined (*14,15*). Using the peak maternal hair mercury level during pregnancy and two end points [the child's neurological examination (Figure 1) and the age they first walked, as reported by the mothers (Figure 2)], a dose–response relationship for prenatal exposure was determined. Using the hockey stick model for dose–response analysis with an estimated background rate of zero in the population, the data for delayed walking gave an estimated lowest effect level of 7.3 ppm with a 95% upper confidence limit of 14 ppm (*15*). If a background rate of 4% was assumed, the estimated lowest effect level was 9 ppm and the upper 95% confidence limit rose to 190 ppm. For neurological signs the same model suggested a background rate of 9% and an estimated lowest effect level of 10 ppm with an upper 95% confidence limit of 287 ppm. Although there was considerable uncertainty in these results, the possibility that prenatal exposures as low as 7–10 ppm in maternal hair might affect brain development was cause for concern. Fish is an important source of protein for many populations around the world and individuals who consume large amounts of fish can have hair mercury concentrations at or above these levels (*1,28*). Because MeHg crosses the placenta, women consuming fish during pregnancy expose their fetus.

There are several concerns about extrapolating data from poisonings such as those that occurred in Iraq to populations consuming fish. Iraq was a poisoning episode with a wide range of doses, some very high, and exposure occurred over a short time period. In contrast, exposure due to fish consumption typically involves repeated small doses for an extended time period. The effects of such differing types of exposure are unknown but may be important (*29*). In addition, the Iraq study had a number of limitations (*14*). The birth dates of the children were not precisely known. The evaluations were limited to a developmental history taken from the mother through an interpreter and a neurological examination done in the child's home. Covariates that could have influenced the child's development were not obtained. Only six children among the mothers with peak hair mercury levels < 50 ppm had abnormal scores on the neurological examination. Three of the mothers had peak hair mercury levels of 1 ppm. The study also included 25 mothers who had peak hair mercury levels > 100 ppm (the highest was 674 ppm).

The statistical uncertainty in the Iraq data was highlighted in two later reports. Cox et al. (*15*) pointed out that there were four influential points in the analysis on delayed development. These points were not outliers in the usual statistical sense, but they had considerable influence on the interpretation of the data and made the estimated threshold difficult to ascertain, as shown in Figure 3.

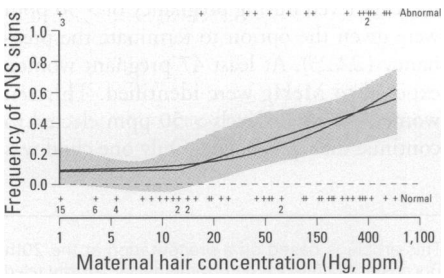

Figure 1. Plots of the logit and hockey stick dose–response analysis of the relationship between central nervous system (CNS) signs and maternal hair MeHg concentrations during gestation. The two dose–response curves are shown by solid lines. The shaded area represents the 95% confidence limits from kernel smoothing. Reproduced from Cox et al. (*15*) with permission of the Academic Press, Inc.

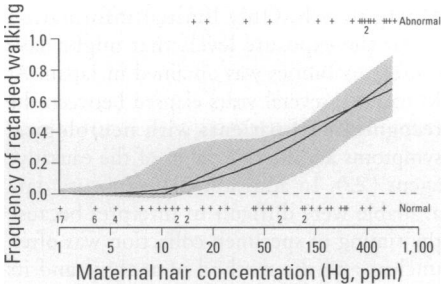

Figure 2. Plots of the logit and hockey stick dose–response analysis of the relationship between retarded walking and maternal hair MeHg concentrations during gestation. The two dose–response curves are shown by solid lines. The shaded area represents the 95% confidence limits from kernel smoothing. Reproduced from Cox et al. (*15*) with permission of the Academic Press, Inc.

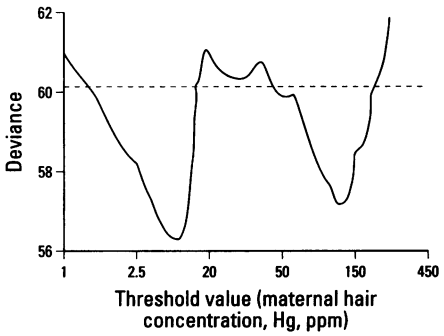

Figure 3. A deviance profile for the threshold parameter of the hockey-stick dose–response model, using the data on delayed walking from Cox et al. (*15*). The abscissa consists of various values for the threshold parameter of the model. The ordinate is the deviance (the goodness of fit is the log likelihood multiplied by –2) of the model with the particular threshold value, and the slope and background response parameters optimized to produce the best fit. The dashed line is used to determine a likelihood based confidence interval for the threshold parameter. Reproduced from Cox et al. (*30*) with permission of Intox Press.

Crump et al. (*31*) also pointed out that thresholds estimated from the Iraq data have considerable statistical variation, are sensitive to how an abnormal response is defined, and are dependent on the model used.

Health Agency Efforts to Establish Safe Levels of MeHg Exposure

Various public health agencies have tried to determine the risk to the general population of MeHg ingestion. In 1972 the World Health Organization established a tolerable weekly intake of 3.3 μg/kg (0.47 μg/kg/day) MeHg (*32*) in the diet, based on data from Japan. The U.S. Environmental Protection Agency subsequently reevaluated the available data and, based primarily on data from Iraq, proposed that the oral reference dose (RfD) be lowered to 0.1 μg/kg/day (*33*). The Agency for Toxic Substances and Disease Registry (ATSDR) reevaluated its profile of MeHg exposure after reviewing data from studies in the Faroe and Seychelles Islands. Based primarily on the Seychelles data (*35*), ATSDR investigators recommended that the minimal risk level (MRL) be set at 0.3 μg/kg/day (*34*). These varying limits of the amount of MeHg that can be safely consumed appear to result from the different studies on which they are based, the uncertainty or safety factor that each agency felt was most appropriate, and differences in definitions (i.e., RfD, MRL, and tolerable weekly intake).

Exposure from Fish Consumption

Because there are many populations around the world that consume large amounts of fish, epidemiological studies were undertaken to determine whether populations frequently consuming fish were indeed at risk. The focus of these studies was on prenatal exposure and its association with child development because the developing brain appears to be most sensitive to the effects of MeHg. Table 1 outlines the studies that have been reported to date. Conclusions from these studies have not been consistent. Some reported an association between prenatal MeHg exposure at levels achieved by fish consumption and the child's scores on developmental tests, whereas others found no association using similar or identical tests. These studies are complex to execute and thus difficult to compare because of differences in study populations, end points measured, covariates assessed, statistical methods utilized, and other factors.

Neurological and Milestone Testing

The two end points associated with poisoning in Iraq, the neurological examination and developmental milestones, have not shown a consistent association with prenatal exposure from fish consumption. A Canadian study of Native Americans who consume fish (*35*) reported an association between the neurological examination and prenatal exposure, whereas two other studies found no association (*36,37*). The Canadian study did not find a consistent dose–response relationship. However, in the highest exposure category (13–23.9 ppm mercury in maternal hair), males did have more abnormal deep tendon reflexes (DTRs) in the neurological examination. Those abnormalities included both increased and decreased DTRs, a finding that the authors caution is difficult to explain physiologically. In addition, changes in DTRs were present only in males. The delays in developmental milestones in Iraq have not been found in populations consuming fish or sea mammals. Studies in Peru (*36*) and the Seychelles Islands (*38*) found normal milestones and a study in the Faroe Islands reported that milestones were achieved early (*39*).

Developmental Testing

A number of studies using more extensive developmental tests than those used in Iraq have been reported. Kjellstrom et al. (*40,41*) from New Zealand reported that the Denver Developmental Screening Test (DDST) and psychological test scores were inversely associated with increasing prenatal MeHg exposure. Marsh (*22*) reviewed the New Zealand reports and concluded that concerns about the study design made it difficult to determine whether an association between the tests and MeHg exposure had been demonstrated. Marsh noted problems in matching both ethnicity and age at testing and the absence of such important covariates as maternal education and intelligence. At 4 years of age the control children were tested at older developmental ages than the cases. However, the DDST is not sensitive or discriminating enough to account for age differences and later testing favored the controls. Marsh also noted that it was a challenging study to analyze because of the social, linguistic, and scholastic differences among the three ethnic groups and that prenatal MeHg exposure accounted for only a small amount (1–2%) of the variance at the 6-year testing.

Subsequently, Crump et al. (*42*) reanalyzed the New Zealand data and reported that the lower limits for prenatal exposure benchmarks on five of the test scores were 17–24 ppm. However, when one child with high exposure (86 ppm) was omitted, the lower limits decreased to 7.4–10 ppm. Other studies of prenatal exposure have also used the DDST and no associations between

Table 1. A summary of epidemiological studies examining the association between prenatal exposure to MeHg from fish consumption and child development.

Study, reference	No.	Prenatal exposure, hair MeHg levels	Outcomes measured	Associations with MeHg exposure
Canada (*35*)	250	Mean 6 ppm Six percent of children with > 20 ppm	Neurological exam DDST	Abnormal DTRs associated with increased MeHg in males with > 13 ppm No dose–response relationship
New Zealand (*40,41*)	290	Mean 4 ppm Peak level 1.5 × mean Range 1–86	DDST Vision, sensory, behavioral, and psychological tests (WISC-III, MSCA)	Levels of > 6 ppm associated with deficiencies on DDST at 4 years Mean level of 13 ppm associated with decreased performance on WISC-III and MSCA at 6 years of age
Peru (*36*)	130	Mean 7 ppm, SD 2 Range 1–28 ppm.	Neurological exam Developmental milestones	No associations reported
Faroe Islands (*45–47,82*)	969	Mean 4 ppm Range (interquartile) 2.6–7.7	Developmental milestones Extensive battery of neuropsychological and neurophysiological tests	No adverse associations were found using hair levels of MeHg Using cord blood mercury adverse associations present with tests of language, attention, memory, visuospatial functions, motor functions, and ABRs at 7 years of age
Seychelles main study (*34,37,38,48,49,83–85*)	779	Median 5.9 ppm Range 0.5–27 ppm	Neurological exam Developmental milestones Extensive battery of psychological tests	Beneficial associations detected No adverse associations were found through the 66-month evaluations

Abbreviations: ABRs, auditory brainstem responses; DDST, Denver Developmental Screening Test; DTRs, deep tendon reflexes; MSCA, McCarthy Scale of Children's Abilities; WISC-III, Wechsler Intelligence Scale for Children-III.

developmental testing and exposure were found (*35,37*). The inconsistency of these reported associations has led to the use of increasingly sophisticated testing methods for evaluating the children.

Grandjean et al. (*44*) reported a study of children in the Amazon basin exposed to MeHg from freshwater fish contaminated after gold mining. The study was cross-sectional and examined 351 children using tests that included finger tapping, the Santa Ana, the Wechsler Intelligence Scale for Children-III, and subtests from the Stanford Binet Intelligence Test. Previous exposure to mercury or other substances was unknown. They reported that test outcomes were adversely associated with the child's hair mercury concentration measured at the time of the testing. They did not have earlier exposure data. They noted that poverty was widespread and that 22% of the mothers consumed alcohol. In the discussion they commented that "test results may have been affected by current tropical diseases or past nutritional deficiencies" (*44*).

The Seychelles and Faroe Islands Studies

Large cohort studies in the Seychelles and Faroe Islands have reported evaluations using extensive test batteries. They reached different conclusions about the association between prenatal MeHg exposure and test outcomes (*34,45,46*).

In the Faroe Islands mercury exposure is mainly from consumption of whale meat. The Faroe Islands study found beneficial associations with mercury exposure and child development during the first year of life that they attributed to breast-feeding (*39*). However, Grandjean et al. (*39*) reported that at 7 years of age there were a number of adverse associations on one or more statistical models between prenatal exposure measured in cord blood and test results (Table 2). These associations were not present when prenatal exposure was measured in maternal hair. The largest adverse effects were associated with attention, learning, and memory, and, to a lesser extent, visuospatial and motor activities (*45,46*). They also reported adverse associations with brainstem auditory-evoked potentials (*47*). The possibility that polychlorinated biphenyls (PCBs), which are present in whale blubber, contributed to the findings was addressed by measuring them in cord tissue and adjusting statistically for this exposure.

In the Seychelles Islands main longitudinal study no adverse associations were present, but statistically significant beneficial associations were found between fish consumption and test outcomes (*34,37,48,49*). Possibly adverse associations were found in the pilot study (*43,50*), but the authors consider the main study more definitive (*34,37,38,48,51*). The main study has more information and tests on the mothers and children, a longitudinal design with evaluations at specific ages, a better testing environment, more covariates including information about the home environment and socioeconomic status, and a larger number of participants. Table 3 outlines the results from the Seychelles studies. Because MeHg is clearly neurotoxic, the enhanced performance as exposure increased in the range being studied may be attributable to other nutrients present in fish. Beneficial effects were present in both sexes on parts of the Preschool Language Scale, the Woodcock-Johnson Test of Achievement, and the Bender Gestalt Test. Crump et al. (*51*) reanalyzed the Seychelles data and reported that the average lower boundary of the benchmark analyses was 25 ppm with a range of 19–30 ppm.

Although the Seychelles and Faroe Islands studies are similar in the intent to study the consequences of prenatal MeHg exposure, they differ in many ways including dietary exposure, covariates, biomarker of exposure, concomitant exposures, testing logistics, outcomes measured, and statistical analysis. The specific factors that account for these differing results have not yet been determined.

MeHg clearly can cause developmental disabilities at sufficiently high doses. Children in Japan had disabilities when industrial pollution resulted in MeHg levels as high as 40 ppm in the fish being consumed (*21*). However, most fresh and saltwater fish have levels of MeHg well below 1 ppm. The associations between exposure and child development that were reported from Iraq and postulated to occur at MeHg levels achieved by consuming oceanic fish have so far not been confirmed and no increase in developmental disabilities has been reported in any study of such a population. Lacking any evidence of an increase in developmental

Table 2. Associations between MeHg and end points from the Faroe Islands studies (main cohort, prenatal exposure).

Test	M	F	Reference
Developmental milestones[a]	B	B	(*39*)
ABR prolonged latencies[b]			
Wave III at 20 and 40 Hz	A	A	(*47*)
Wave I–III at 20 Hz	A	A	
Wave V	A	A	(*45*)
Finger Tapping[b]			
Preferred hand	A	A	(*45*)
Both hands	A	A	(*46*)
Hand–Eye Coordination[b]			
Average of all trials	A	A	(*46*)
CPT[b]			
Missed responses	A	A	(*45*)
Average reaction time	A	A	
WISC-R[b]			
Digit span	A	A	(*45*)
Similarities	A	A	
Block design (square root)	A	A	
Bender Gestalt[b]			
Errors in copying	A	A	(*45*)
Reproduction	A	A	
Boston Naming Test[b]			
No cues	A	A	(*45*)
Cues	A	A	
California Verbal Learning Test[b]			
Short-term reproduction	A	A	(*45*)
Long-term reproduction	A	A	

Index of exposure, maternal hair mercury levels for prenatal exposure at 1 year of age and cord blood for prenatal exposure at 7 years of age. Abbreviations: A, adverse association on one or more analysis; ABR, auditory brainstem responses; B, beneficial; CPT, Continuous Performance Test; F, females; M, males; WISC-R, Wechsler Intelligence Scale for Children-Revised.
[a]1 year of age. [b]7 years of age.

Table 3. Associations between MeHg and end points from the Seychelles Islands studies.

Cohort, age	Test	Exposure	Males	Females	Reference
Main					
Birth	Birth weight	Prenatal	B	–	[a]
19 months	Enhanced BSID-MDI with increasing MeHg exposure in higher caregiver IQ groups at several levels of family income	Prenatal	B	B	(*49*)
29 months	BSID-IBR (activity)	Prenatal	B/A	–	(*48*)
66 months	PLS (total Score)	Prenatal	B	B	(*34*)
	W-J (applied problems)	Postnatal	B	B	
	Bender Gestalt (errors)	Postnatal	B	–	
Pilot					
5–109 weeks	DDST	Prenatal	A	A	(*43*)
66 months	PLS (auditory comprehension)	Prenatal	A	A	(*50*)
96 months	Boston Naming Test	Prenatal	B	–	(*85*)
	Beery-Butenka (VMI)	Prenatal	B	–	(*85*)
	Grooved Pegboard				
	Preferred hand	Prenatal	B	A	(*85*)
	Nonpreferred hand		B	–	

Index of exposure, maternal hair mercury levels for prenatal exposure and for child at 5 years of age for postnatal exposure. Abbreviations: A, adverse; B, beneficial; B/A, unclear if beneficial or adverse; BSID, Bayley Scales of Infant Development; CPT, Continuous Performance Test; DDST, Denver Developmental Screening Test; GCI, General Cognitive Index; IBR, Infant Behavior Record; MDI, Mental Developmental Index; PLS, Preschool Language Scale; VMI, Visual Motor Integration (like Bender); W-J, Woodcock-Johnson Test.
[a]Not published.

disabilities, researchers have examined the possibility of subtle developmental changes being present. Increasingly complex test batteries and sophisticated study designs have been used to address this possibility. However, a consistent pattern of evidence that exposure to small amounts of MeHg from fish consumption is associated with subtle developmental deficits is not yet available.

Important Issues That Need Resolution

How Should MeHg Exposure Be Determined?

Accurately measuring the magnitude, duration, and timing of exposure, including how much reaches the target organ, is important when trying to test associations with outcomes. Mercury levels have generally been measured in hair or blood. In both hair and blood it is possible to measure total mercury, MeHg, and inorganic mercury. Total mercury in both hair and blood correlate well with MeHg exposure and with brain levels (*52,53*). Most clinical studies have used maternal hair growing during pregnancy to determine prenatal MeHg exposure (*19,34, 35,40,41,45*). One study suggested that cord blood mercury levels may be preferable (*45,54*). Because the brain is the target organ and can be measured only postmortem, the measure that best reflects brain levels would seem preferable.

When MeHg is ingested, it is almost totally absorbed from the gastrointestinal tract and enters the blood stream. In blood it is mostly bound to hemoglobin in the red cells. From blood, it readily crosses the placenta, the blood–brain barrier, and enters hair follicles. In the hair follicle it is incorporated into the hair shaft as it grows. The total mercury concentration in hair, blood, and brain are directly correlated (*1,52,53*). Transport of MeHg across the blood–brain barrier and into the brain is on an amino acid carrier and transport into the hair follicle is thought to be by a similar mechanism (*55*). Thus, hair measurements may provide an indirect noninvasive method of measuring MeHg levels in the brain. Hair is not a reliable biomarker for inorganic mercury or mercury vapor (*56*). Mercury vapor does not appear to be deposited in the growing hair. Hair grows at a fairly uniform rate and can be analyzed segmentally. In this way exposure can be recapitulated for a time period limited only by the hair length. However, more research into the relationships among blood, hair, and brain mercury levels after MeHg exposure, transport into hair follicles, and other issues of transport, binding, and degradation is needed.

Determination of prenatal MeHg exposure has been reported using total mercury levels in maternal hair growing during pregnancy and levels in cord blood. Which measure most accurately reflects fetal brain exposure is controversial. In blood the half-life of MeHg is approximately 50 days and measured levels can vary substantially depending on recent dietary exposure (*1,29,57*). Consequently, blood levels provide accurate information only about recent exposure. Cord blood would not be expected to reflect exposure that occurs earlier in pregnancy. The Faroe Islands study measured mercury in cord blood taken at delivery, as well as maternal hair growing during the pregnancy (*45*). Grandjean et al. (*45,46*) reported an adverse association between cord blood levels and the children's test results at 7 years of age, but these associations were not present using maternal hair mercury levels during pregnancy. Based on these findings, the authors argued that cord blood might more accurately reflect exposure (*46,54*). Measuring cord blood would not identify exposure earlier in pregnancy. Measuring maternal hair growing during the pregnancy by segmental analysis can detect exposures throughout the entire pregnancy. It is not clear if cord blood, which measures a fraction of the gestational exposure, or maternal hair, which can provide information about exposure during the entire pregnancy, actually provides a better measure of fetal exposure.

Do Peak or Mean Mercury Levels Reflect Brain Exposure More Accurately?

When large doses of MeHg are ingested, as with the Iraq poisoning, spikes or peaks are present in blood and hair (*19*). These peaks can be detected by serial measurements of hair or blood. Peak hair mercury values were used in determining the prenatal dose–response relationships found in Iraq (*15*). Individuals who consume marine mammals such as whales may also have peaks. These animals contain high levels of mercury and maximum consumption may occur irregularly and in fairly large amounts when meat is available. Most species of fish have substantially lower levels of MeHg and individuals who consume fish regularly are more likely to be in a steady state. Although variations may occur in their mercury levels, significant elevations or peaks are less likely to be present or may be much less pronounced. If adverse effects are associated with peak exposure, the mean exposure may not be the best measure. Most epidemiological studies of populations consuming fish have used the mean hair mercury value during pregnancy as the primary measure of exposure. Peak mercury values were associated with neurological signs and developmental milestones in Iraq, but with lower exposures over long periods of time it is unclear which value most accurately reflects brain exposure.

Should the Biomarker for Recent High-Level and Chronic Low-Level Exposures Be Different?

Blood levels obtained close to the time of exposure provide an accurate determination of acute exposures. However, with human exposure it may not be possible to obtain samples immediately. At Minamata it was several years after exposure before MeHg was suspected, as the cause and the level and duration of exposure were not well defined. In Iraq, MeHg poisoning was recognized early and both blood and hair levels were helpful. Blood levels were useful with acutely ill patients, whereas hair levels were helpful in recapitulating exposure after the outbreak. When dealing with exposures in individuals who regularly consume seafood, the levels are substantially lower and they may be in a steady state with only minor variations in mercury level. The biomarker that best reflects brain exposure after fish consumption needs to be determined.

Does Dietary Exposure to Low Doses of MeHg Differ from High-Dose Exposure?

Dietary exposure is generally to small amounts of MeHg over prolonged periods of time, whereas high-dose exposure more often occurs over shorter time intervals. There may be differences between how the human body detoxifies or excretes mercury depending on the type of exposure. Clarkson (*29*) proposed that the liver may detoxify small amounts of MeHg but be unable to handle larger amounts.

Dietary sources often contain nutrients that have beneficial effects or may decrease the toxicity of MeHg. Many fish are rich in selenium and long-chain polyunsaturated fatty acids, especially those termed omega-3 fatty acids. Selenium may decrease the toxicity of MeHg, and omega-3 fatty acids are important in brain development (*1,58*). The beneficial effects of omega-3 fatty acids may outweigh any adverse effects of the MeHg.

What Is the Importance of Exposures at Different Ages?

Most epidemiological studies have studied prenatal exposure, but postnatal exposure may also be important. Infants consume breast milk that can contains MeHg; toddlers and older children consume fish and fish products. Although the most serious effects of MeHg appear to be on cell migration and differentiation, processes that are most active prenatally, brain development continues at a slower pace for years postnatally. A limited number of associations between postnatal exposure and outcomes have been reported. Amin-Zaki et al. (*9,11,13*) reported postnatal poisoning in Iraq. Both the Faroe and Seychelles Islands studies examined postnatal exposure. The

Faroe Islands study reported that breast-feeding was associated with enhanced achievement of early developmental milestones (*39*). However, Grandjean et al. (*45*) reported no associations between postnatal exposure measured at 1 and 7 years and an extensive test battery given at 7 years of age. In the Seychelles Islands beneficial associations were reported between MeHg exposure measured in children's hair postnatally at 66 months and several test outcomes (*34*). Further study of the contribution of postnatal MeHg exposure to developmental outcomes is needed.

How Should Studies Account for Concomitant Exposures?

Before attributing any association to MeHg, it is important to be certain that other pollutants such as lead, PCBs, organochlorines, etc., have not contributed to the results. Unfortunately, many human exposures are to multiple environmental contaminants, making the contribution of individual contaminants more difficult to define. For example, the flesh of marine mammals may contain both MeHg and inorganic mercury, and individuals who consume the flesh often consume the blubber, which may contain other contaminants. Accurately measuring these environmental contaminants in the appropriate biological tissue and adequately considering them in the analysis is essential to excluding them as contributing factors.

Other compounds of mercury should also be considered concomitant exposures. Many individuals including children have dental amalgams that release small amounts of mercury vapor. Animal studies have suggested that exposure to mercury vapor can cause developmental problems in rats and primates (*59,60*). Experimental work also suggests that animals exposed to both MeHg and mercury vapor may experience greater toxicity (*61*). Data on concomitant human exposure to MeHg and mercury vapor both prenatally and postnatally are needed.

Are Animal and Human Exposures Comparable?

There is a large body of data from animal studies that consistently indicates associations between exposure to low dosages of MeHg and developmental abnormalities. These studies span a wide range of species from rodents to primates. MeHg exposure affects functions ranging from operant conditioning to visual discrimination (*62,63*). Some tests used in animals to detect low levels of exposure (*64,65*) have been used in children and no association was found (*37*).

One difficulty in comparing animal and human studies is how to define low-level exposure. In 1990 Burbacher et al. (*62*) reviewed the MeHg literature and compared animal and human data. They defined low-level exposure as one where mercury measured in the brain was < 3 ppm. Using this definition, the authors found low-level exposure reports of neuropathological changes in small mammals but not in primates or humans. They also reported neurobehavioral effects in humans and small mammals at low level but not in primates. The authors considered reports of impaired performance on operant tasks in small mammals and delayed developmental milestones in children to be low-level exposures even though brain levels were not actually measured in those studies (*15,66*). However, Burbacher et al. (*62*) commented that "... subtle effects do not provide many similarities across species."

The lowest brain mercury levels associated with either neurobehavioral or neuroanatomic changes in animals are approximately 1,800 ppb (*67,68*). In comparison, human fetal brain levels from maternal fish consumption are reportedly < 300 ppb (*69*). The exposure Burbacher et al. (*62*) defined as low is approximately 6 times the exposure level for infants whose mothers consume fish regularly. Until similar exposures are studied in humans and animals, comparisons will be difficult.

Measuring exposure in animal experiments is frequently done differently than in human studies. In experimental animals a known dose of a single toxin is given so the exposure is well known or can be accurately measured either during life or when the animal is sacrificed. However, the level of mercury in brain or hair is seldom measured or reported. In comparison, in human populations the exposure is rarely known, must be determined by measuring levels in some biological tissue such as hair or blood, and is sometimes to more than one substance.

Physiological differences between humans and experimental animals also present challenges. In primates the blood half-life of mercury is reportedly shorter than in humans and there are substantial differences in the ratio of mercury between blood and brain in various species (*70,71*). Studies of primates exposed to doses of MeHg prenatally and postnatally or just postnatally have provided evidence for impairment of visual, auditory, and vibratory sensation, cognitive and social development, and operant behavior (*71,72*). There is also evidence of delayed neurotoxicity that appeared only with aging (*73*). However, the brain level of MeHg exposure associated with these effects has not been reported.

How Important Is Effect Modification?

Bellinger (*74*) pointed out that factors correlated with exposure or development may modify the association between the exposure to a neurotoxin and its effects on development. Nutritional factors comprise one group of such effect modifiers that are particularly pertinent to MeHg exposure from dietary sources. Breast milk is believed to have many beneficial effects for infant development (*75*). It contains nutrients such as docosahexanoic acid, taurine, and cholesterol that may not be present in infant formulas or cow's milk. However, breast milk can also contain small quantities of organic and inorganic mercury if maternal exposure is high, and it can be an important source of exposure (*9,10,13*).

Grandjean et al. (*39*) reported that children in the Faroe Islands achieved developmental milestones early. The authors found an association between milestone achievement, breast-feeding, and the level of MeHg measured in the infant's hair at 12 months of age. As both hair mercury concentration and length of breast-feeding increased, the age at achieving milestones decreased. They reasoned that MeHg could not be responsible for this effect; thus, there must be a beneficial effect from breast-feeding. The association between breast milk and MeHg exposure was not explored further in that study. Explaining this outcome may be complex. The modification of the association between MeHg and development may not have been only on the outcome variable. Nutrients in breast milk might have somehow truncated the neurotoxic effect of MeHg. Experiments in mice have shown that selenium deficiency enhances the adverse effects of fetal MeHg exposure on neurobehavior (*76*).

It may also be important whether the source of the MeHg exposure is from consuming fish, marine mammals, or both. There are both similarities and differences between fish and marine mammals. Both contain significant quantities of selenium and amino acids, but marine mammals more often contain other chemicals that may adversely affect a child's development, such as PCBs, dioxins, and organochlorines. The quantity of MeHg in marine mammals can be as high as 3 ppm, a level above that found in most fish (*77*), and substantial amounts of inorganic mercury are often also present in sea mammals.

Social and cultural factors such as maternal intelligence [intelligence quotient (IQ)] and socioeconomic status (SES) may also modify the association between exposure and developmental outcomes. Bellinger (*74*) argued that SES may actually influence the effect of lead on child development, with greater effects at lower levels of SES. In the Seychelles Child Development Study main cohort, family income and caregiver IQ were also found to act as effect modifiers (*49*). Children whose caregiver had a higher IQ were more likely to show enhanced cognitive development with higher mercury levels at 19 months compared to those children whose caregiver had a lower IQ score. The role of these effect modifiers appears to be important, but is not yet well defined.

How Should Child Development Be Evaluated?

Studies of developmental outcomes in children after maternal exposure to MeHg have used standardized measures of development, including assessment of developmental milestones and standardized psychological tests and measurements. In some cases, the test batteries have been developed from a theoretical model predicting domains of development believed vulnerable to MeHg exposure (*78,79*). In other cases, specific neuropsychological functions have been examined (*80*). Recently, the ATSDR adopted recommendations from a panel of experts on pediatric neurobehavioral testing in environmental health field studies (*81*). This battery uses both screening tests and standardized psychological measures.

Despite the wide variety of available approaches to assessment, there is no test or measurement for which specificity or sensitivity to MeHg effects has been documented. When high-dose exposures are being assessed and severe outcomes such as mental retardation and cerebral palsy are expected, this lack of performance data is not critical. However, little is known about expectations for adverse developmental outcomes when exposures are at the lower limits of toxicity. Most researchers have predicted that the effects after low-dose exposure would be subtle. In the absence of sensitivity and specificity data for MeHg, the interpretation of results may depend on other variables that may affect developmental outcomes. Thus, studies that report adverse effects do so based on the assumption that they are due to MeHg and not other factors.

What Are the Problems in Conducting a Statistical Analysis?

A number of issues need to be considered in the analysis of data from any observational study of human exposure. Is the study exploratory or confirmatory? Exploratory studies are designed to generate hypotheses, which must then be confirmed in additional studies. In contrast, confirmatory studies examine human populations to see if effects that have been hypothesized to exist are actually present. Consequently, confirmatory studies are generally held to a higher level of statistical rigor than exploratory studies. With MeHg there was a large body of experimental work in animals and some preliminary studies from human poisonings in Japan and Iraq that suggested low levels of exposure might adversely affect child development. Studies of MeHg exposure in populations consuming fish are thus confirmatory in nature.

Given multiple end points, sufficient analyses, and the possibilities of statistical analysis, evidence for nearly any hypothesis can be supported. Therefore, the development of an analysis plan is essential. This plan should be in place before the data are examined and should not be modified as analysis proceeds. The design should include a statement of how exposure will be measured, a listing of primary outcome variables, and a list of covariates to be used in the primary analysis. The plan should also describe the statistical models to be used to assess the relationship between exposure and outcome and each end point should be examined individually. Secondary hypotheses can be addressed in later analyses.

The plan should include tests to determine if the assumptions made by the statistical model being used are in fact true. In models based on multiple linear regression, a technique widely used to analyze data from environmental studies, the critical assumptions require independent normally distributed errors with constant variance and linearity of relationships between independent variables and outcome. Standard methods are available to check these assumptions and this should be part of the analysis plan. The data should also be checked for statistical outliers, points that are inconsistent with the statistical model, and influential points—points that may not be outliers but which have a disproportionate influence on the results of the analysis.

For confirmatory studies a limited number of specific end points should be chosen so that statistical associations between exposure and outcomes can be interpreted as causal relationships. This does not appear to be the case in many studies of the effects of environmental toxins on child development. In the absence of a limited number of sensitive and specific outcome variables it is tempting to use a large battery of tests so that any possible effect will be identified. The problem with such a battery is that the various tests may not be very specific and that statistical multiplicity (type I errors) may make the results of many analyses difficult to interpret.

Summary

Exposure to high levels of MeHg can result in developmental disabilities. However, the associations among exposure, developmental milestones, and neurological tests that were originally reported from Iraq and postulated to occur at MeHg exposure levels as low as 10 ppm have been difficult to confirm. In populations where exposure is from consuming oceanic fish, both beneficial and adverse associations have been reported. The beneficial associations are believed to be due to other nutrients in fish. Increasingly sophisticated study designs, testing, and statistics have been incorporated into the research looking for subtle clinical effects of MeHg exposure from fish consumption. However, no consistent pattern of adverse effects has been found and no scientific agreement on any adverse associations has yet been reached. Although poisoning with MeHg can cause developmental disabilities, the evidence that lower levels of exposure contribute to disabilities is presently limited.

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